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Computational Approaches to the Identification of Suspect Toxic Molecules

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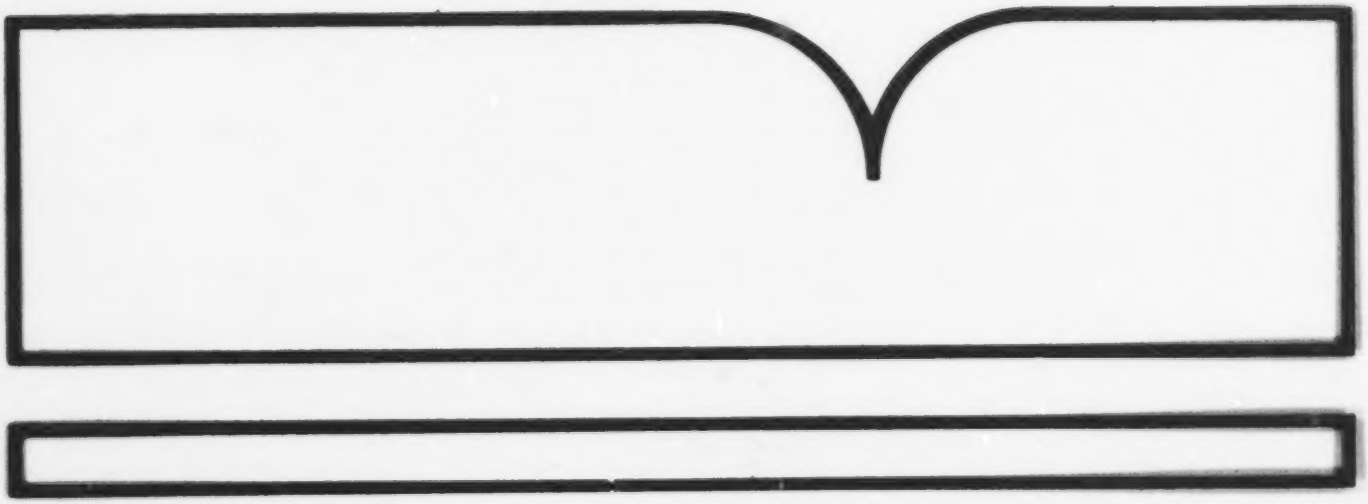

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16. ABSTRACT

We have presented computational approaches that can be used for the relatively rapid identification of suspect toxigens, including carcinogens, in two different classes of compounds: (a) halogenated olefins and epoxides, and (b) substituted dibenzo-*p*-dioxins. A common element in these approaches is the key role played by the molecular electrostatic potential. It is applied in two different ways, however; it is used to assess the reactivity of a specific site in the case of the epoxides, and for the dibenzo-*p*-dioxins the focus is on the overall pattern of negative regions above the molecular plane. While we are continuing to develop and refine both types of analysis, especially that related to the dibenzo-*p*-dioxins, the results obtained so far are encouraging, and indicate that these can be regarded as useful screening techniques for identifying compounds that require further and more exhaustive investigation.

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Computational approaches to the identification of suspect toxic molecules

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SUMMARY

We have presented computational approaches that can be used for the relatively rapid identification of suspect toxigens, including carcinogens, in two different classes of compounds: (a) halogenated olefins and epoxides, and (b) substituted dibenzo-*p*-dioxins. A common element in these approaches is the key role played by the molecular electrostatic potential. It is applied in two different ways, however; it is used to assess the reactivity of a specific site in the case of the epoxides, and for the dibenzo-*p*-dioxins the focus is on the overall pattern of negative regions above the molecular plane. While we are continuing to develop and refine both types of analysis, especially that related to the dibenzo-*p*-dioxins, the results obtained so far are encouraging, and indicate that these can be regarded as useful screening techniques for identifying compounds that require further and more exhaustive investigation.

INTRODUCTION

Certain classes of chemicals (e.g., polynuclear aromatic hydrocarbons, halogenated hydrocarbons, dibenzo-*p*-dioxins) are found on a relatively large scale in our environment. Not all of the individual compounds of these types pose a serious health threat. Since some do, however, there is a need for fast but reliable techniques for assessing the relative degrees of hazard associated with specific members of these classes. One possible approach involves animal testing. This is a lengthy and expensive undertaking, however, and the results are very sensitive to the conditions of the testing procedure.

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Accordingly, one of our objectives has been to develop effective alternatives that complement current laboratory approaches, and thus avoid the unrealistic prospect of having to apply the latter to hundreds of compounds. We need to formulate rapid and reliable computational procedures for assessing and predicting degrees of toxicity so that the likely hazard associated with a compound can be estimated in a matter of hours. Animal testing, which requires extended periods of time, could then be focused only on those compounds that already have been shown to be suspect.

We have shown, for two different classes of chemicals, that the molecular electrostatic potential provides a basis for the initial assessment of toxicity. This is the potential $V(r)$ that is created in the space around a molecule by its nuclei and electrons; it is through this potential that the molecule is 'seen' or 'felt' by any other system in its vicinity. The value of $V(r)$ at any point r is given rigorously by Eqn. (1):

$$V(r) = \sum_A \frac{Z_A}{|r_A - r|} - \int \frac{\varrho(r')dr'}{|r' - r|} \tag{1}$$

where Z_A is the charge on nucleus A, located at R_A, and $\varrho(r)$ is the electronic density function of the molecule, which we obtain from the computed molecular wave function.

The first term on the right side of Eqn. (1) gives the contribution of the nuclei, which is positive; the second represents the effect of the electrons, which is negative. Thus, when an electrophile approaches the molecule, its initial tendency is to go to those regions where $V(r)$ has its most negative values, since it is there that the effects of the molecule's electrons predominate. A knowledge of the electrostatic potential around a molecule is therefore of considerable assistance in interpreting its reactive behavior and in predicting the sites on the molecule at which interactions are most likely to occur [1–3].

A second important application of the electrostatic potential is in the area of 'recognition' processes such as occur in enzyme-substrate or drug-receptor interactions. In such a process, the initial step is one of recognition: the receptor 'recognizes' that an approaching molecule has certain key features that will promote their mutual interaction. Such recognition is believed to take place typically when the molecule and the receptor are at a relatively large separation; it precedes the formation of any covalent bonds.

Since the electrostatic potential of a molecule is a physically meaningful representation of how it is perceived by a system in its vicinity, it is reasonable to look to $V(r)$ in seeking the key features that determine whether or not a recognition process involving the molecule and a particular cellular receptor will occur. The potential has indeed proven to be an effective means for analyzing and elucidating recognition processes; in a number of cases (which are reviewed in Politzer et al. [4]), the affinity of a given molecule for a specific receptor has been shown to depend upon the degree to which the potential of the former possesses certain characteristics that

have been established as being required for effective interaction with that receptor.

An important feature of the electrostatic potential is that it is a real physical property, which can be determined experimentally by diffraction methods as well as computationally [3]. At present, however, the latter approach is by far the more widely used.

We used the electrostatic potential to determine the susceptibilities of certain molecular sites toward electrophilic attack and to identify general patterns of positive and negative potential that promote interaction with a specific cellular receptor. Each approach has been found to be effective for assessing the likely toxicities of individual members of a particular class of compounds.

Halogenated olefins and their epoxides

The halogenated olefins and their epoxides are a widely occurring and commercially important class of chemicals. They are used in the manufacture of textiles and plastics, as solvents, pesticides, dry-cleaning fluids, refrigerants, flame retardants, degreasing agents, and as intermediates in syntheses. At least a dozen halogenated olefins have been identified in drinking water [5,6]. (For a detailed discussion of the occurrence of halogenated olefins and epoxides in the environment, see Woo et al. [7].)

Many of these compounds are known to be carcinogenic. Perhaps the most notorious is vinyl chloride, which has been shown to cause liver angiosarcomas, lung adenocarcinomas, and other tumors in animals [8,9], and has been strongly implicated in liver, lung, and lymphatic cancers in humans [10,11]. Other halogenated olefins and epoxides are also known to be carcinogens; some of these include epichlorohydrin, *cis*- and *trans*-1-chloropropylene oxide, *cis*- and *trans*-1,3-dichloropropylene oxide, vinylidene fluoride and vinylidene chloride, vinyl bromide, ethylene oxide and propylene oxide, and others [7,12–15].

There appear to be at least four important factors involved in the carcinogenic behavior of halogenated olefins and epoxides.

(1) Ease of epoxidation of the olefin

The initial step in the metabolism of many halogenated olefins is epoxidation, which occurs through the action of microsomal monooxygenases [7,13,14,16–18]. It is the resulting epoxides that are believed to be responsible for the carcinogenicities of a number of these compounds [7,14,19,20]. If this metabolic epoxidation involves interaction with an electrophilic oxygen species [21], then it should proceed more readily as the electrostatic potential in the olefinic double-bond region becomes more strongly negative. For the chlorinated ethylenes, we have shown that this potential becomes more negative as the number of chlorines decreases [22], which parallels a general increase in reactivity of these molecules toward electrophiles that has been observed experimentally [23–26].

(2) Tendency of the epoxide to undergo oxygen protonation

Protonation of an epoxide oxygen is known to weaken the C–O bonds and facilitate the opening of the epoxide ring [27–30]. We have further evidence of this in terms of C–O force constants and bond lengths [31–33]. For example, protonation of the epoxide oxygen typically lengthens the C–O bonds by about 0.07 Å.

Supporting the expectation that the halogenated olefin epoxides are protonated in the environment of interest is the fact that there do exist subcellular regions with high proton activities [34–36]. The nuclear membrane, in which the enzymatic reactions of DNA replication occur, contains certain redox systems that are believed to produce high local proton acitivities [36–38], including the cytochrome *P*-450 system that epoxidizes olefins [39].

(3) Reactivity of the protonated epoxide

The epoxide needs to be sufficiently reactive to undergo the interaction(s) involved in the carcinogenic process. However, too great a degree of activity leads to a high likelihood of its reacting with other cellular species before reaching the key site(s) at which the carcinogenic process would be initiated. (This happens in the case of the *syn* and *anti* 7,8-dihydrodiol-9,10-epoxides of benzo[a]pyrene; the *syn* is chemically more active while the *anti* is more carcinogenic [40–43].) The concept that there is some zone of intermediate reactivity which is optimum for initiating the carcinogenic process has been expressed earlier [33,44–47].

(4) Nature of the critical cellular adduct that is formed

If and when the potentially carcinogenic cellular interaction occurs, presumably involving DNA, it can be expected that the properties and behavior of the product will help to determine the subsequent course of events. Recently, the primary in vivo DNA alkylation product of vinyl chloride has been shown to be the 7-*N*-(2-oxoethyl) derivative of guanine [48,49]. The carcinogenicity of vinyl chloride has been attributed to the formation of this derivative. Its NMR spectrum was interpreted as indicating that it may exist in equilibrium with a cyclic hemiacetal [48]; this would affect the hydrogen bonding between guanine and cytosine, and could lead to miscoding [50–52] and replicational and transcriptional errors [48,53]. We have carried out computational analyses of systems designed to model the oxoethyl derivative and the hemiacetal [54]; we estimate the equilibrium to be very far in the direction of the former.

Since the carcinogenic process appears to involve a number of separate steps, it must be anticipated that it will not be possible to establish a direct correlation between the carcinogenicity and any single molecular property or parameter. However, we have found a criterion that does permit us to separate epoxides into the two general categories of (1) likely to be carcinogenic, and (2) likely to be inactive or only weakly carcinogenic. This criterion, which will now be discussed in detail, appears to be related to the second of the four factors mentioned above (i.e., the tendency of the epoxide to undergo oxygen protonation).

Computational approach

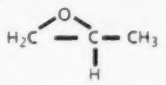
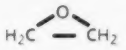
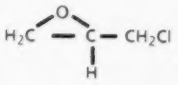
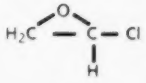
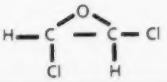
As was pointed out above, it is the metabolically produced epoxides that are believed to be the carcinogenic forms of the halogenated olefins. Accordingly, our computational analyses have focused upon epoxides. For each one that we have studied, the first step was to calculate its optimized geometry. This was done even in those instances when an experimentally determined structure was available, in order to ensure consistency in comparing the results for different molecules. An ab initio self-consistent-field (SCF) molecular orbital procedure was used at the STO-3G level; this basis set has been shown to be effective for structure calculations [55]. Once the geometry had been obtained, the molecular electrostatic potential was computed, in terms of the STO-5G basis set. We have found this to yield good results for properties related to the electronic charge distribution, as is $V(r)$.

Oxygen potentials as a criterion for epoxide carcinogenicity

Protonation and oxygen potentials

Epoxides typically have negative electrostatic potentials in the neighborhood of the oxygen [56,57]. There are generally two local minima (points at which the most negative values are found) that can be viewed as reflecting the oxygen's lone pairs.

TABLE 1. COMPARISON OF CALCULATED PROTON AFFINITIES AND V_{min} VALUES [a,b]

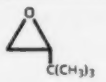
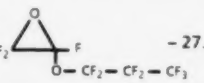
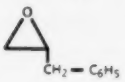
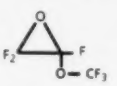
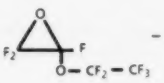
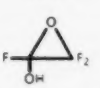
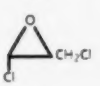
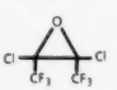
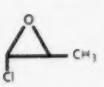
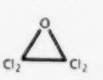
Name of Molecule	Structure	Proton Affinity (Oxygen)	V_{min} (Oxygen)
Propylene Oxide		239	- 53.4
Ethylene Oxide		232	- 51.3
Epichlorohydrin		226	- 43.1
Chlorooxirane		217	- 38.1
trans-Dichlorooxirane		204	- 23.1

a The proton affinities and the V_{min} values were computed at the STO-3G and STO-5G levels, respectively. All are in kcal/mole.

b The data in this table are taken from Politzer et al. [47].

Table 1 compares the calculated proton affinities of several epoxides (oxygen protonation) with the single most negative value of $V(r)$ in the vicinity of each oxygen, denoted V_{min}. The excellent correlation between V_{min} and the proton affinity demonstrates that V_{min} is a good indicator of the relative ease of protonation of these molecules.

TABLE 2. CALCULATED V_{min} VALUES (KCAL/MOLE) FOR SOME EPOXIDES[a]

	Molecule	V_{min} (Oxygen)		Molecule	V_{min} (Oxygen)		Molecule	V_{min} (Oxygen)
1.		−54.0	12.		−40.3	22.		−28.4
2.		−53.9	13.		−39.9	23.		−27.7
3.		−53.4	14.		−39.8	24.		−27.6
4.		−53.2	15.		−38.1	25.		−26.7
5.		−51.3	16.		−34.8	26.		−26.6
6.		−51.2	17.		−34.2	27.		−26.5
7.		−47.7	18.		−33.2	28.		−23.1
8.		−46.5	19.		−33.1	29.		−17.1
9.		−43.1	20.		−31.5	30.		−15.6
10.		−40.8	21.		−29.5	31.		−9.2
11.		−40.7						

[a] The data in this table are taken in part from Politzer and Laurence [57].

Since protonation is an important factor in facilitating epoxide reactions, as discussed above, we have computed V_{min} for a large number of epoxides. The results are presented in Table 2. The magnitudes of the oxygen minima clearly can be related to the nature of the substituent on the epoxide ring. Thus, the substitution of a halogen on ethylene oxide causes V_{min} to become less negative, presumably due to the halogen attracting electronic charge away from the oxygen. The effect increases with the degree of halogenation and is stronger when the substitution is directly on the ring than when it involves an exocyclic carbon. Chlorine affects the oxygen potential more than does fluorine, despite the latter's greater intrinsic electronegativity. This again demonstrates the anomalous nature of fluorine [58,59], which can be interpreted in terms of Huheey's concept of charge capacity [60–62]: the ability of fluorine to accommodate additional electronic charge diminishes relatively rapidly, despite the strong initial attraction. Finally, Table 2 shows that aliphatic hydrocarbon groups cause V_{min} to become slightly more negative than it is in ethylene oxide, which is consistent with the weakly electron-releasing natures of these groups [27,63].

Enzyme inhibition

Since V_{min} directly reflects the electron-withdrawing or -releasing powers of substituents on epoxide rings, it seemed reasonable to explore its use in developing a quantitative measure of the abilities of epoxides to inhibit epoxide hydrase, an enzyme that catalyzes the hydrolysis of epoxides. This inhibition is often due to the inhibitor competing as an alternate substrate [64–66], and thus should depend upon factors similar to those believed to determine the extents of interaction of epoxides with epoxide hydrase; these include the electron-withdrawing or -releasing tendencies of substituents on the epoxide ring and steric compatibility [66]. V_{min} is a measure of the former; to take steric effects into account, we used Taft's steric substituent parameter E_S [67,68]. In confirmation of this reasoning, we were indeed able to demonstrate a good correlation between epoxide hydrase inhibition and the quantity V_{min}/E_S [56]; the correlation coefficient is 0.94.

A criterion for the identification of suspect carcinogens

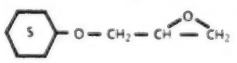
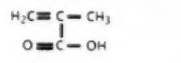
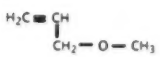
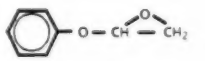
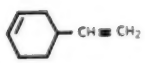
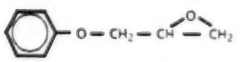
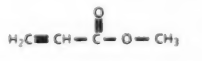
We have found that there is a general relationship between the electrostatic potential and carcinogenicity for hydrocarbon and halogenated hydrocarbon epoxides. More specifically, we have shown that carcinogenicity is associated with a relatively strong negative potential in the neighborhood of the epoxide oxygen [56].

When the epoxides are ranked in order of their V_{min} values, the established carcinogens (as shown by laboratory studies) are found to have the more negative V_{min}; the inactive or weakly active ones have less negative values. The threshold potential, in terms of our STO-5G computational level, is approximately -30 kcal/mol. (This would probably change somewhat if another basis set were used, which simply means that it is necessary to be consistent with respect to the basis set.)

These observations provide a useful predictive capability for the identification of suspect carcinogens. If the electrostatic potential near the oxygen of a halogenated hydrocarbon epoxide reaches values more negative than roughly −30 kcal/mol, then the molecule should be regarded as reasonably likely to be carcinogenic. In the practical application of this criterion, it is an important advantage that the magnitude of V_{min} follows well-defined patterns related to the natures of the substituents on the epoxide ring, as pointed out earlier. Once these patterns have been discerned through computational analyses of a large number of molecules (Table 2), V_{min} often can be estimated for other systems to within 1–2 kcal/mol, without the necessity for further calculation. For example, for the epoxide of 3-chloro-2-methylpropene, our prediction was that V_{min} would be between −42 and −45 kcal/mol; its computed value turned out to be −42.9 kcal/mol.

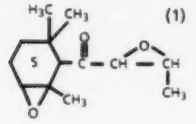
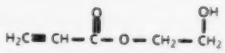
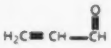
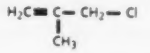
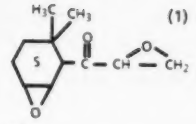
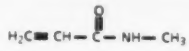
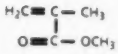
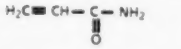
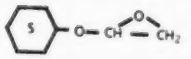
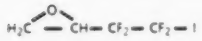
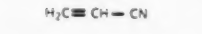
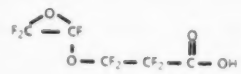
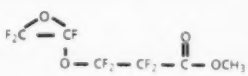
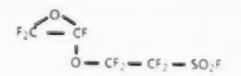
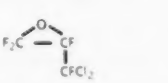
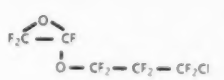
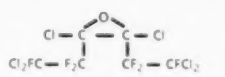
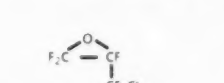
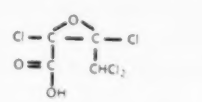
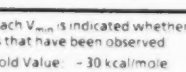
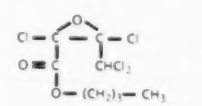
We suggest that the apparent association of carcinogenicity with a strongly negative oxygen potential reflects the importance of protonation at that site. This was mentioned earlier as one of four factors that appears to be involved in the carcinogenic behavior of halogenated olefins and epoxides. However, while a strongly negative V_{min} seems to be a prerequisite for carcinogenicity, its magnitude does not indicate the degree of activity. For example, vinyl chloride is certainly more carcinogenic than either ethylene or ethylene oxide [7], yet V_{min} for chlorooxirane (the active metabolite of vinyl chloride) is −38.1 kcal/mol, compared to −51.3 kcal/mol for ethylene oxide (Table 2). In order to be able to estimate the relative carcinogenicities of the epoxides, it is necessary to be able to take quantitative account of their overall reactivities. We are currently addressing this problem [69].

TABLE 3. CALCULATED AND ESTIMATED V_{min} VALUES FOR OLEFINS AND EPOXIDES AS REQUESTED BY ENVIRONMENTAL PROTECTION AGENCY. FOR OLEFINS, V_{min} IS FOR THE CORRESPONDING EPOXIDE.[a,b]

Molecule	V_{min} (kcal/mole)	Molecule	V_{min} (kcal/mole)
	−54 (est.)		−46.3 (calc.)
	−53.3 (calc.)		−45.7 (calc.)
	−53.3 (calc.)		−44.8 (calc.)
	−53 (est.)		−44.2 (calc.)

(continued)

TABLE 3. (Continued).

Molecule	V_{min} (kcal/mole)	Molecule	V_{min} (kcal/mole)
 (1)	(1) – 51 (est.) (2) – 49 (est.)		– 43.4 (calc.)
(2) 	– 49.6 (calc.)		– 42.9 (calc.)
 (1)	(1) – 49 (est.) (2) – 47 (est.)		– 39.9 (calc.)
(2)			– 39.9 (calc.)
	– 48.9 (calc.)		– 39.3 (calc.)
	– 47 (est.)		– 39 (est.)
	– 46.5 (calc.)		– 35.9 (calc.)
	– 25.5 (calc.)		– 34.8 (calc.)
	– 25 (est.)		– 29.5 (calc.)
	– 25 (est.)		– 21.8 (calc.)
	– 25 (est.)		– 16 (est.)
	– 25 (est.)		– 16 (est.)
	– 24.4 (calc.)		– 16 (est.)

[a] Beside each V_{min} is indicated whether it was calculated directly (at the STO-5G level) or estimated on the basis of the patterns that have been observed.

[b] Threshhold Value: – 30 kcal/mole.

Application of the V_{min} criterion

We have applied our electrostatic potential criterion for carcinogenicity, involving V_{min}, to a number of compounds that the Environmental Protection Agency requested us to analyze. These were not limited to the categories of halogenated olefins and their epoxides, although all of them are either epoxides of some sort or contain double bonds that can presumably form epoxides metabolically. Out of a total of 32 compounds, we found 23 to be suspect carcinogens (Table 3), for which further evaluation is suggested.

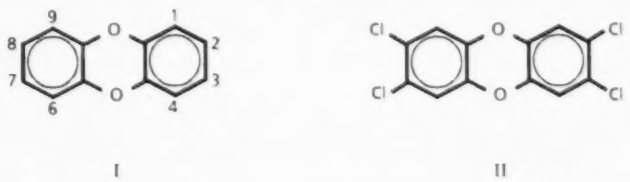
Dibenzo-p-dioxins

Dibenzo-*p*-dioxin (Fig. 1, I) is the parent compound for a large number of derivatives, with halogens and other substituents at various positions. These exhibit varying degrees of toxicity, ranging from virtually none to very high, the latter being exemplified by the notorious 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, Fig. 1, II).

These compounds are formed as trace contaminants in the manufacture of herbicides, insecticides, fungicides, and disinfectants. They are also produced in very small amounts in combustion processes, including the burning of wood.

The toxic responses produced by the dibenzo-*p*-dioxins include, to varying degrees, gastric and liver lesions, hepatomegaly, loss of lymphoid tissue, urinary tract hyperplasia, acute loss of weight, and chloracne [70,71]. Relatively few of these compounds have been tested for carcinogenicity; however, TCDD (Fig. 1, II) has been shown to be a highly potent carcinogen in mice and rats [72,73], and it has also been found to act as a tumor promoter [74]. A mixture of two hexachlorodibenzo-*p*-dioxins was observed to be carcinogenic in rats and mice [75], whereas the 2,7-dichloro derivative is only marginally active [76] and unsubstituted dibenzo-*p*-dioxin is noncarcinogenic [77].

In addition to the various degrees of toxicity exhibited by the dibenzo-*p*-dioxins, certain other biochemical responses are elicited as well. One of these is the induction of aryl hydrocarbon hydroxylase (AHH) activity [78,79]. This enzyme is responsible for the hydroxylation and epoxidation of polycyclic aromatic hydrocarbons [39]. An excellent correlation between the toxicities of the dibenzo-*p*-dioxins and their



I II

Fig. 1. Structural formulae of dibenzo-*p*-dioxin (I) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (II).

potencies in inducing AHH activity has been found [71,78–82]; for example, the highly toxic TCDD is also an extremely strong AHH-inducer.

These observations suggest that some mechanistic feature(s) may be common to both the toxic and the AHH-inducing activities of these compounds. Indeed, it is now believed that both the toxicity and the AHH induction involve initial binding of the molecule to the same cytosolic receptor [71,83,84]. Both the toxicities and the AHH-inducing potencies have been found to correlate well with binding affinities to this receptor.

Certain structural features of the dibenzo-*p*-dioxins have been identified as being associated with high degrees of toxic potency, AHH induction and binding to the cytosolic receptor [71,79]. These are as follows:

1) The molecule should be essentially planar and rectangular, with dimensions that are roughly 3 × 10 Å.
2) At least three of the four lateral positions (2, 3, 7, and 8) should have halogen substituents. The activity is greater if all four of these positions are halogenated.
3) The activity induced by halogen substituents decreases in going from bromine to chlorine to fluorine.
4) For activity, at least one ring position should remain unsubstituted.

It was later suggested that the need for an unsubstituted position reflects a steric requirement of the receptor for an unhindered region of space [85,86]. It was further proposed that AHH induction may require activation of the receptor by an interaction between a negative center on the molecule (e.g., a chlorine) and an electrophilic site on the receptor.

In view of the evidence indicating that the toxic effects produced by dibenzo-*p*-dioxins are related to their binding to a cytosolic receptor, our work has focused upon this interaction. We treat this as involving a recognition process, and accordingly seek patterns in the electrostatic potentials of the dibenzo-*p*-dioxins that can be related to their degrees of toxicity.

Computational approach

We have computed the SCF STO-5G electrostatic potentials of molecules I through XII [87,88]. The structure of TCDD(II) has been determined crystallographically [89], and this was used as the model for the other systems, with appropriate modifications: chlorines were replaced by X (X = H, CH_3, or F) with C–C–X angles set at 120°. C–H and C–F bond lengths were taken to be 1.08 Å and 1.35 Å, respectively [90, 91], while the $-CH_3$ geometries were taken from an optimized toluene structure [92]. For each molecule, $V(r)$ was calculated at 1.75 Å above the molecular plane, since this is what a receptor would encounter; 1.75 Å is the van der Waals' radius of chlorine [93], which is the largest atom found in these molecules. $V(r)$ was also computed in the perpendicular plane through the oxygens to determine the effects of various degrees of substitution upon the negative potentials near the oxygens.

Table 4 presents the most negative values (the local minima) of $V(r)$ in the plane 1.75 Å above each of the molecules I through XII, as well as the minima near the oxygens, obtained from the perendicular plots. (Figures showing the calculated electrostatic potentials of I, II, IV, and IX through XII are given by Murray et al. [87] and Murray and Politzer [88].)

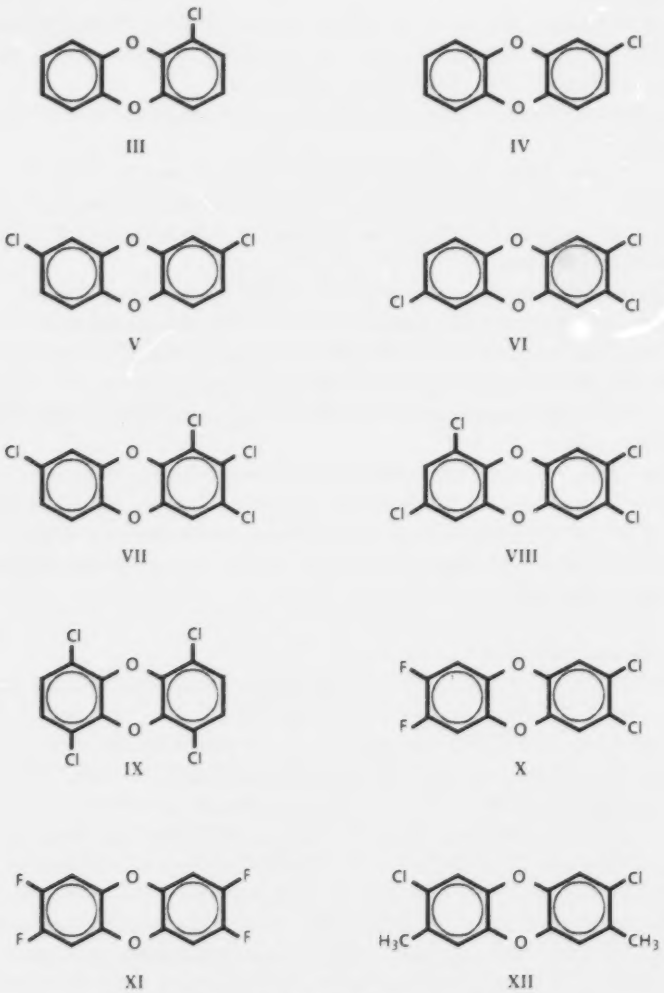
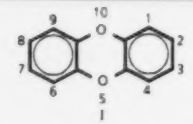
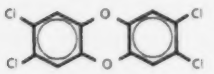
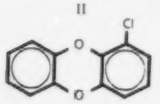
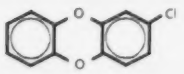
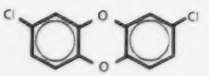
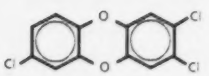
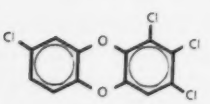
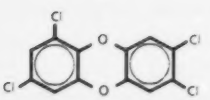
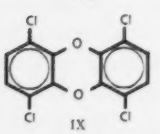


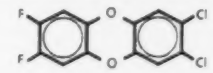
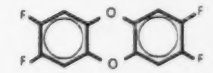
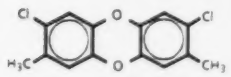
Fig. 2. Structural formulae of dibenzo-*p*-dioxins.

TABLE 4. CALCULATED ELECTROSTATIC POTENTIAL MINIMA OF SOME DIBENZO-*p*-DIOXINS.

Molecule	V(r) minima at 1.75 A above molecular plane (kcal/mole)	V(r) minima near oxygens, in perpendicular plane (kcal/mole)
 I	− 11.6 (0)[a] − 5.8 (aromatic rings)[a]	− 50.7[a]
 II	− 12.9 (Cl)[a]	− 28.9[a]
 III	− 20.5 (Cl) − 7.2 [0(5)]	− 50.4 [0(10)] − 45.5 [0(5)]
 IV	− 18.6 (Cl) − 7.3 [0(10)] − 6.8 [0(5)]	− 45.5 [0(10)] − 44.0 [0(5)]
 V	− 16.4 (Cl) − 3.3 [0(10)] − 2.1 [0(5)]	− 39.4 [0(10)] − 38.7 [0(5)]
 VI	− 14.9 [Cl(2)] − 14.8 [Cl(3)] − 14.4 [Cl(7)]	− 34.2 [0(10)] − 35.5 [0(5)]
 VII	− 12.5 [Cl(1)] − 13.5 [Cl(2)] − 11.4 [Cl(3)] − 13.4 [Cl(8)]	− 34.6 [0(10)] − 29.6 [0(5)]
 VIII	− 13.7 [Cl(2)] − 13.3 [Cl(3)] − 10.5 [Cl(7)] − 11.3 [Cl(9)]	− 34.5 [0(10)] − 29.6 [0(5)]
 IX	− 14.8 (Cl)	− 40.1

(continued)

TABLE 4. (continued).

Molecule	V(r) minima at 1.75 A above molecular plane (kcal/mole)	V(r) minima near oxygens, in perpendicular plane (kcal/mole)
 X	−15.1 (Cl) −3.2 (F)	−34.7
 XI	−5.3 (F) −3.6 (O)	−40.6
 XII	−16.8 (Cl) −5.2 [O(10)] −3.4 [O(5)]	−42.8 [O(10)] −41.1 [O(5)]

Only the parent, unsubstituted molecule (I) has negative regions above the aromatic rings; these can be interpreted as being due to the π electrons. Molecule I also has strong negative potentials near each oxygen, with minima above and below the molecular plane that can be attributed to its lone pairs; this is typical of what has been found for oxygens in other systems. (See Table 2 and Politzer and Laurence [57].) These potentials extend well beyond the plane 1.75 Å above the molecule.

It is notable that the electron-withdrawing effect of just one chlorine substituent suffices to eliminate the negative regions above the aromatic rings, even for the ring that does not bear the chlorine (see results for III and IV in Table 4). The introduction of chlorines also produces a significant weakening of the oxygen negative potentials; the $V(r)$ minima go from -50.7 kcal/mol for the unsubstituted system I to -28.9 kcal/mol for the tetrachloro derivative II. The spatial extents of these negative regions also decrease; thus, whereas they still extend beyond the 1.75 Å plane in the dichlorinated molecule (V), the substitution of a third chlorine, in VI, reduces their sizes to the point that no negative potentials remain at 1.75 Å (Table 4). (IX appears to be an exception to these trends; however, its anomalously strong oxygen potentials are due to the proximity of the chlorines and the consequent overlapping of the oxygen and chlorine negative regions.)

Table 4 shows that there are invariably negative potentials above the chlorine and fluorine substituents. These become weaker as the number of halogens increases, reflecting the fact that each is receiving a smaller share of the polarizable electronic charge. This is a phenomenon that we have observed in other systems in the past [22,88]. The data in Table 4 again bring out the fact that chlorine is better able to accommodate additional electronic charge than is fluorine. This can be seen, for ex-

ample, by comparing the results for II and XI. Even more striking is the observation that the chlorines in X are more negative than their counterparts in either II or VI, showing that they compete more successfully for the polarizable electronic charge against two fluorines than against even only one chlorine (as in VI).

Among the molecules that have been included in this study, I, III through V, IX, XI, and XII are biologically relatively inactive [71,78]. Table 4 shows that it is also these molecules that have the strongest and most extensive negative potentials associated with the oxygens. We have accordingly suggested that biological activity on the part of the substituted dibenzo-*p*-dioxins may require that the negative potentials above the oxygens be considerably weaker than in the parent molecule, I [87,88].

We have also pointed out that the presence of relatively strong negative potentials above most or all of the two lateral regions of the molecule (positions 2, 3 and 7, 8) appears to be linked to biological activity [87,88]. Thus, molecules VI through VIII, all of which show some degree of activity [71,78], have negative potentials of between -10 and -15 kcal/mol above one lateral region and half of the other (Table 4), while in the highly active TCDD (II), the potentials are in this range above all of both lateral regions. In contrast, most of the inactive or very weakly active molecules do not have even one lateral region that is completely negative; the exception is XI, but its lateral potentials are very weak.

On the other hand, it should not be inferred that the degree of biological activity necessarily becomes greater as the lateral regions become more negative. In II, VI, and VIII, activity increases [79,85] as the lateral potentials become less negative. This suggests that there may be some intermediate range of values that corresponds to a high level of activity.

In conclusion, our studies have focused attention upon the electrostatic potentials above the lateral regions and the oxygens of the substituted dibenzo-*p*-dioxins [87,88]. Biological activity appears to require negative potentials above all or most of the lateral regions, with an apparent optimum range of magnitudes in the neighborhood of -13 kcal/mol (when computed with the STO-5G basis set). A second requirement seems to be a very significant weakening of the negative potentials associated with the oxygens.

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